

ELISA PROTOCOL

IgG₁/IgG_{2a}



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Used by the Laboratory of William C. Gause, Ph.D.

STEP	mL/WELL	DILUTION	BUFFER	INCUBATION
Coat with Capture Antibody - Purified α-mouse IgG IgG ₁ - Binding Site Cat # PC273 IgG _{2a} - Binding Site Cat # PC274	50	1:1400	BBS	2 hrs. RT Overnight 4°C
Wash Plates 3 times	300		ddH ₂ O	
Block the Plate	50		PCS	30 min. RT
Wash Plates 3 times	300		ddH ₂ O	
Apply Standards and Samples Standard - Start with 625 ng/mL and do 1:3 serial dilutions Samples - Start with 1:10,000 and do 1:3 serial dilutions	50		BBS + Tween	2 hrs. RT Overnight 4°C
Wash Plates 3 times	300		ddH ₂ O	
Incubate with Wash Buffer	50		BBS + Tween	30 min. RT
Wash Plates 3 times	300		ddH ₂ O	
Incubate with Detection Antibody - α-mouse IgG-AP IgG ₁ - Southern Biotechnology Associates Cat # 1070-04 IgG _{2a} - Southern Biotechnology Associates Cat # 1080-04	50	1:2000	BBS + Tween	30 min. RT
Wash Plates 3 times	300		ddH ₂ O	
Incubate with Wash Buffer	50		BBS + Tween	30 min. RT
Wash Plates 3 times	300		ddH ₂ O	
Add Substrate and Develop - MUP MUP - Sigma Cat # M-8883	50	5mg/100mL	MUP Buffer	Until Highest Standard Reaches 2800 F.U.
Detect Fluorescence on ELISA Plate Reader				

ELISA protocol developed using Immulon II plates from Dynex, Cat #3455.

Manufacturer Phone Numbers:

1. The Binding Site Limited – 0121.471.4197
2. Southern Biotechnology Associates, Inc. – 205.945.1774
3. Sigma – 800.325.3010
4. Dynex – 703.631.7800



IgG₁/IgG_{2a} ELISA Buffers

1. Borate Buffered Saline (BBS)

Begin with 5L ddH₂O in 6L Ehrlenmeyer flask.

Add the following slowly with stirring:

H₃BO₃ : 61.8 g

NaCl : 43.8 g

Adjust pH to 8.5 with 1N NaOH.

Bring volume to 6L with ddH₂O.

Store at Room Temperature (RT).

2. BBS + Tween

Add 10mL of 1% Tween-20 stock to 500mL BBS.

Store at Room Temperature.

3. Protein Carrier Solution (PCS)

Add 5mL Fetal Bovine Serum to 500 mL BBS

Add 5mL 10% Sodium Azide.

Store at 4°C.

4. MUP Buffer

Begin with 5L ddH₂O in 6L Ehrlenmeyer flask.

Add 25.2g NaHCO₃ and 1.21g MgCl₂ • 6H₂O

Adjust pH to 9.7 with 1N NaOH.

Bring volume to 6L with ddH₂O.

Store at Room Temperature (RT).